MICROFICHE

013397

MEMORANDUM

SUBJECT: Methyl Bromide -- Combined chronic/oncogenicity feeding - rat (83-5),MRID 4462501. Barcode 242525; S. 536414; Chem. No. 053201; Rereg. Case # 0335. Toxicology Chemical No. 555.

TO: Robert McNally/Joseph Nevola PM 60.
SRRD (7508C)

FROM: Stanley B. Gross, PhD, DABT, CIH
Registration Action Branch 3, HED (7509C) 5/24/99

THRU: Stephen Dapson, PhD 5/26/99
Branch Senior Scientist, Registration Branch 3, HED (7509C)

Attached is the Data Evaluation Record and study for:

CITATION: Mertens, J.J.W.M. (1997) A 24-month chronic dietary study of methyl bromide in rats. WIL Research Laboratories, Inc.,1407 George Road, Ashland, OH 44805-9281, Laboratory Study No. WIL-49014, December 9, 1997, MRID 44462501. Unpublished

EXECUTIVE SUMMARY:

In a chronic toxicity/oncogenicity study (MRID 44462501), microencapsulated methyl bromide was administered to 4 groups of male and female Crl:CD®(SD)BR rats for a period of 12 or 24 months (interim and main study, respectively) in the diet at concentrations of 0 (diet control), 0 (placebo control), 0.5, 2.5, 50, or 250 ppm. These concentrations resulted in doses of 0, 0.02, 0.11, 2.20, and 11.10 mg/kg/day in males and 0, 0.03, 0.15, 2.92, and 15.12 mg/kg/day in females for the controls, 0.5, 2.5, 50, and 250 ppm groups, respectively. Groups of 50 males and 50 females were designated for the main study and were maintained on the treated food for up to 104 weeks. Groups of 20 males and 20 females were sacrificed at 52 weeks in the diet control, placebo control, 50 ppm group, and the 250 ppm group.

Survival was not affected by the test substance in any of the treated groups compared to either of the control groups. No treatment-related clinical signs or effects on hematology, serum chemistry, urinalysis, or organ weight data were observed. The test article did not produce changes in ophthalmoscopic examinations for the treated groups compared to the controls. Macroscopic and microscopic evaluations of organs and tissues at the interim and final sacrifices revealed only normal age-related changes, changes that were observed with equal frequency in the controls, and/or were sporadic and not dose-related. Treatment with methyl bromide did not produce oncogenicity when fed to rats for up to 2 years.

Statistically significant treatment-related effects were observed on body weights, body weight

gains, and food consumption in males and females treated with 250 ppm of the test substance during the first 12 to 18 months of the study. Males in the 250 ppm group had decreases of 5.5% in mean body weight compared to the diet control at week 2, by week 14 this decrease was 10% and remained consistently lower through week 70, during the second year of the study these animals gradually regained the weight and were comparable to controls at the end of the study. Females in the 250 ppm group had a decrease of 3.7% in mean body weight compared to the diet control at week 2, by week 14 this decrease was 8.3% and also remained consistently lower through week 57. After week 57 females in the 250 ppm group gained weight gradually and the decreases disappeared by the end of the study (week 104) at which time this group had mean body weight values that were similar to controls. Mean body weight gain was markedly decreased during the first 18-months of the study for animals treated with 250 ppm methyl bromide; decreases of 9-18% and 12-21% were observed for males, and 7-22% and 11-19% were observed for females when compared to the basal diet and placebo control groups, respectively. Males receiving 250 ppm had decreased food consumption that ranged from 3.7 - 11.5 % for week 71-72, and females at this concentration had decreases of 4.8 - 10.5% for week 54-55 compared to their respective control groups.

The LOAEL is 250 ppm (11.10 mg/kg/day for males and 15.12mg/kg/day for females), based on decreased body weight, body weight gain, and food consumption in males and females during the first 18 months of the study. The NOAEL is 50 ppm (2.20 mg/kg/day for males and 2.92 mg/kg/day for females).

No evidence of carcinogenicity was observed in male or female rats fed Methyl Bromide at dietary concentrations of 0.5, 2.50, 50 or 250 ppm for 104 weeks. Dosing was adequate based on decreases in body weight, body weight gain, and food consumption in males and females.

This chronic toxicity/carcinogenicity study in the rat is **Acceptable/guideline** and satisfies the guideline requirement for a combined chronic toxicity/carcinogenicity oral study (§83-5) in rats.

If you have any questions concerning the study review, please contact Stan Gross 305-6382.

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DATA EVALUATION REPORT

METHYL BROMIDE

STUDY TYPE: COMBINED CHRONIC/ONCOGENICITY FEEDING - RAT (83-5)

MRID 44462501

013397

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 99-16

Primary Reviewer: Tessa L. Long, Ph.D.	Signature: Date:	MAR 2 6 1999
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	MAR 2 6 1999
Robert H. Ross, M.S. Group Leader	Signature: Date:	MAR 2 6 1999
Quality Assurance: Bobbette D. Nourse, Ph.D.	Signature: Date:	3/29/99

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed by Lockheed Martin Energy Research Corporation for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464

EPA Primary Reviewer: Stanley B. Gross, Ph.D. Stanley Gross, Date 5/17/99
Registration Action Branch 3 (7509C)
EPA Secondary Reviewer: Ayaad Assaad, D.V.M, Ph.D. _____, Date 5/24/99
Registration Action Branch 3 (7509C)

013397

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/oncogenicity feeding – Rat
OPPTS 870.4300 [§83-5]

DP BARCODE: D242525
P.C. CODE: 053201

SUBMISSION CODE: S536414
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Methyl Bromide (0.48% and 3.44% purity)

SYNONYMS: bromomethane; curafume; embafume; haltox; iscobrome; monobromomethane; terabol; Brom-O-Sol; Brom-O-Gas; Meth-O-Gas; Terr-O-Gas; Brom-O-Gaz; Celfume; Kayafume; MeBr; Halon 1001; Dowfume MC-2; Dowfume MC-33; EDCO; MB; MBX; Metafume; methogas; profume; Rotox; terr-o-gas 100; Zytox (Ref. ChemID 1999)

CITATION: Mertens, J.J.W.M. (1997) A 24-month chronic dietary study of methyl bromide in rats. WIL Research Laboratories, Inc.,1407 George Road, Ashland, OH 44805-9281, Laboratory Study No. WIL-49014, December 9, 1997, MRID 44462501. Unpublished

SPONSOR: Methyl Bromide Industry Panel, Chemical Manufacturers Association
1300 Wilson Boulevard, Arlington, VA 22209

EXECUTIVE SUMMARY:

In a chronic toxicity/oncogenicity study (MRID 44462501), microencapsulated methyl bromide was administered to 4 groups of male and female Crl:CD®(SD)BR rats for a period of 12 or 24 months (interim and main study, respectively) in the diet at concentrations of 0 (diet control), 0 (placebo control), 0.5, 2.5, 50, or 250 ppm. These concentrations resulted in doses of 0, 0.02, 0.11, 2.20, and 11.10 mg/kg/day in males and 0, 0.03, 0.15, 2.92, and 15.12 mg/kg/day in females for the controls, 0.5, 2.5, 50, and 250 ppm groups, respectively. Groups of 50 males and 50 females were designated for the main study and were maintained on the treated food for up to 104 weeks. Groups of 20 males and 20 females were sacrificed at 52 weeks in the diet control, placebo control, 50 ppm group, and the 250 ppm group.

Survival was not affected by the test substance in any of the treated groups compared to either of the control groups. No treatment-related clinical signs or effects on hematology, serum chemistry, urinalysis, or organ weight data were observed. The test article did not produce changes in ophthalmoscopic examinations for the treated groups compared to the controls. Macroscopic and microscopic evaluations of organs and tissues at the interim and final sacrifices revealed only normal age-related changes, changes that were observed with equal frequency in the controls, and/or were sporadic and not dose-related. Treatment with methyl bromide did not produce oncogenicity when fed to rats for up to 2 years.

Statistically significant treatment-related effects were observed on body weights, body weight gains, and food consumption in males and females treated with 250 ppm of the test substance during the first 12 to 18 months of the study. Males in the 250 ppm group had decreases of 5.5% in mean body weight compared to the diet control at week 2, by week 14 this decrease was 10% and remained consistently lower through week 70, during the second year of the study these animals gradually regained the weight and were comparable to controls at the end of the study. Females in the 250 ppm group had a decrease of 3.7% in mean body weight compared to the diet control at week 2, by week 14 this decrease was 8.3% and also remained consistently lower through week 57. After week 57 females in the 250 ppm group gained weight gradually and the decreases disappeared by the end of the study (week 104) at which time this group had mean body weight values that were similar to controls. Mean body weight gain was markedly decreased during the first 18-months of the study for animals treated with 250 ppm methyl bromide; decreases of 9-18% and 12-21% were observed for males, and 7-22% and 11-19% were observed for females when compared to the basal diet and placebo control groups, respectively. Males receiving 250 ppm had decreased food consumption that ranged from 3.7 - 11.5 % for week 71-72, and females at this concentration had decreases of 4.8 - 10.5% for week 54-55 compared to their respective control groups.

The LOAEL is 250 ppm (11.10 mg/kg/day for males and 15.12mg/kg/day for females), based on decreased body weight, body weight gain, and food consumption in males and females during the first 18 months of the study. The NOAEL is 50 ppm (2.20 mg/kg/day for males and 2.92 mg/kg/day for females).

No evidence of carcinogenicity was observed in male or female rats fed Methyl Bromide at dietary concentrations of 0.5, 2.50, 50 or 250 ppm for 104 weeks. Dosing was adequate based on decreases in body weight, body weight gain, and food consumption in males and females.

This chronic toxicity/carcinogenicity study in the rat is **Acceptable/guideline** and satisfies the guideline requirement for a combined chronic toxicity/carcinogenicity oral study (§83-5) in rats.

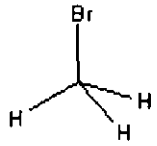
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Microencapsulated Methyl Bromide, received from Midwest Research Institute, Kansas City, Missouri in two formulations.

   Description: white powder
   Lot/Batch No.:
   Formulation 1: CMA-MBIP 29.0 MICRO-MRI, MRI-REFER NO. 357901, 0.48% Methyl Bromide WIL log no. 2600A
   Formulation 2: MRI project no. 357901 CMA Reference no. 29.0-Micro-MRI, 3.44%; Methyl Bromide, WIL log no. 2773A
   Purity: 0.48% and 3.44% for Formulations 1 and 2, respectively
   Stability of compound: -15 °C ± 5 °C
   Formulation 1, 0.48% : 12 months 99.5%, 24 months 80.0%
   Formulation 2, 3.44% : 12 months 106.0%, 24 months 97.0%
   CAS #: 74-83-9
   Stucture:



http://chemfinder.camsoft.com/

2. Vehicle and/or positive control

   The test substance was administered in basal diet (PMI Feeds, Inc.© Certified Rodent LabDiet©). The placebo microcapsules (control) were obtained from Midwest Research Institute, Kansas City, Missouri. No positive control was used.

3. Test animals
   Species: Rat
   Strain: Crl:CD® (SD)BR
   Age and weight at study initiation: approx. 7 weeks; weight: males: 155–247 g; females: 126–188 g
   Source: Charles River Laboratories, Inc., Portage, MI
   Housing: singly in wire mesh cages above cage board
   Diet: PMI Feeds, Inc.© Certified Rodent LabDiet© 5002 was available *ad libitum*, except during the fasting periods before blood collection
   Water: Municipal water was available *ad libitum*
   Environmental conditions:

Temperature: 70 - 74°F
Humidity: 15.6 - 82.8 %
Air changes: not given
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 21 days

B. STUDY DESIGN

1. In life dates

Start: February 23, 1995 end: February 26, 1997

2. Animal assignment

At the end of the acclimation period animals that were deemed suitable for use in the study by the study director based on general physical state and weight were assigned by computer randomization to the test groups in Table 1.

TABLE 1: Study design

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg/day)		Main Study (24 months)		Interim Sacrifice (12 months)[a]	
		male	female	male	female	male	female
1	0 (Basal Diet)	0	0	50	50	20	20
2	0 (Empty Microcapsule)	0	0	50	50	20	20
3	0.5	0.02	0.03	50	50	0	0
4	2.5	0.11	0.15	50	50	0	0
5	50	2.20	2.92	50	50	20	20
6	250	11.10	15.12	50	50	20	20

Data taken from pp. 20 and 26, amd 43, MRID 44462501.
[a] For the interim sacrifice, 20 rats/sex/group (1,2,5,6) were treated for 52 weeks then sacrificed

3. Dose selection rationale

The study author reported that dose selection was based on the results of a previously conducted range-finding study, WIL-49015 (1996) A Two Week Dietary Range-Finding Toxicity Study of Methyl Bromide in Rats. WIL Research Laboratories, Inc., Ashland, OH. Details were not provided.

4. Diet preparation and analysis

Appropriate amounts of the test article to be added to the basal diet for the respective concentration groups were determined using a correction factor of 208.3 for the 0.5 and the 2.5 ppm groups, a correction factor of 29.1 was used for the 50 and 250 ppm groups. Beginning May 15, 1995 (week 11) test article used for groups 3 and 4, the 0.48% formulation of methyl bromide, was filtered prior to use in order to reduce the variability of concentration distribution for these groups. For the placebo control diet, the weight was equilibrated to the microencapsulated methyl bromide for the 250 ppm group. The appropriate amount of placebo or microencapsulated methyl bromide was weighed and transferred to a Patterson-Kelly® twin shell dry blender with a small amount of rodent diet, where the mixture was blended for ten minutes. Appropriate amounts of feed were then added to this premix to make the desired target diet concentrations, this batch was mixed for 15 minutes in order to achieve a homogeneous diet for each test group. Diets were prepared for each test group once per week and immediately frozen until use, at which time the diets were thawed before administration.

Results -

Homogeneity Analysis: Four homogeneity analyses were conducted for samples collected from the top, middle, and bottom of the blender from diets which were prepared on 1-31-95 and 2-7-95 (pre-initiation), 3-31-95, 5-16-95, and 4-23-96. These analyses revealed that the mean % concentrations for the 0.50 ppm group ranged from 90.6 - 106 % of the target concentration, 89.2 - 113 % for the 2.5 ppm group, 96.0 - 110.0 % for the 50 ppm group, and 95.0 - 100 % of the target concentration for the 250 ppm group.

Stability Analysis: Stability of the test substance was assessed at room temperature after 16 or 24 hours and frozen after 8 or 14 days, and after three weeks. The concentration of methyl bromide in the dietary samples after storage at room temperature for 16 hours were 71.4, 66.2, 93.0, and 89.0% of the time zero concentration for the 0.50, 2.5, 50, and 250 ppm groups respectively. The concentration of methyl bromide after 24 hours at room temperature was 63.2, 72.0, 86.3, and 86.6 % of the time zero concentration for the 0.50, 2.5, 50, and 250 ppm groups respectively. After storage for 8, 14, or 21 days the 0.5 ppm group concentrations were 72.4, 146, and 91.8 %, respectively and the 2.5 ppm group concentrations were 102, 124, and 95.8 % of the time zero concentration, respectively. After 8 and 14 days the concentrations were 87.1 and 99.3 %, respectively for the 50 ppm group and the 250 ppm group remained at 103% of the baseline concentration after either 8 or 14 days storage at -20°C.

Concentration Analysis: The overall mean concentrations of the test article present in the rodent diet preparations were 0.495 ppm (0.50 ppm group, 99.0%), 2.53 ppm (2.5 ppm, 101%), 50.8 ppm (50 ppm, 102%), and 257 ppm (250 ppm, 103%). The

individual formulations that were outside the 15 % acceptability range for the target dose were either reanalyzed and found to be within this range or were not used for test administration.

Conclusions: The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. The stability data indicate that the samples were for the most part stable for the required durations in this study and the actual concentration summary indicated that there was less than 15% variation of target concentration.

5. Statistics

The following parameters were analyzed using the one-way analysis of variance followed by Dunnett's Test: body weight, body weight change, food consumption, clinical pathologic data, and absolute and relative organ weights.

The survival of treated groups and both control groups was compared for the chronic and oncogenicity phase animals using the RXC Chi-square Test.

Tumor incidences at certain time points or time intervals were analyzed by the Fisher exact test for comparison of treated groups with controls.

All tests were two-sided. Statistical significance was set at 1 or 5%.

C. METHODS

1. Observations

Animals were inspected before the start of the study for physical and behavioral abnormalities and twice daily for signs of moribundity and mortality. Detailed clinical observations were conducted weekly, this included inspections for the presence of palpable masses.

2 Body weight

Animals were weighed weekly beginning two weeks prior to the study, immediately before study initiation and weekly thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/rat/day for each cage. Mean compound intake (mg/kg/day) values were calculated from food consumption and body weight gain or body weight data.

4. Ophthalmoscopic examination

The eyes of all animals were examined before study initiation, and the eyes of all animals in the main study that survived to week 51 and study termination (week 103) were also examined. The eyes were examined using a binocular indirect ophthalmoscope following mydriasis.

5. Blood was collected from the tail vein of the 10 lowest numbered animals/sex/group at 3, 6, 12, 18, and 24 months for hematology and clinical chemistry analysis. Animals were fasted before collection, and blood smears were collected for those animals euthanized *in extremis*. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet estimate*	X	Reticulocyte count
	Blood clotting measurements*		Red cell distribution width
X	Activated partial thromboplastin time	X	Red cell morphology
	Clotting time		
X	Prothrombin time		

* Required for chronic studies and chronic toxicity/oncogenicity based on Subdivision F Guidelines

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins (by calculation)
X	Sodium*	X	Albumin/Globulin Ratio
		X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein*
X	Alkaline phosphatase (Alkalinephosphatase)		Triglycerides
X	Aspartate amino-transferase* (Aspartattransfer)		Serum protein electrophoresis
X	Alanine aminotransferase* (Alaninetransfer)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic toxicity and chronic toxicity/oncogenicity studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected from the same animals used for clinical chemistry analysis. The animals were fasted during urine collection. The CHECKED (X) parameters were examined.

X		X	
X	Appearance* and color	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin
X	pH	X	Occult Blood*
X	Sediment (microscopic)*	X	Nitrites
X	Protein*	X	Urobilinogen
		X	Leukocytes (WBC)

*Required for chronic toxicity and chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.

7. Sacrifice and pathology

All animals surviving to scheduled termination (week 52, interim or week 104, termination) as well as those found dead or euthanized *in extremis*, were subjected to

gross pathological examination. These animals were anesthetized with carbon dioxide and exsanguinated. The CHECKED [X] tissues from main study and interim sacrifice animals were collected and stored in 10% neutral buffered formalin for histological examination. These tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Microscopic evaluation of the preserved tissues from the 0.5, 2.5, and 50 ppm groups was limited to the lungs, brain, eyes (optic nerve), stomach, liver, kidneys, masses and gross lesions. The [XX] organs from all animals surviving to scheduled sacrifice were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	X	Adrenal gland*
X	Colon*	XX	Kidneys*+		Harderian gland
X	Rectum*	X	Urinary bladder*		Lacrimal gland
XX	Liver*+	XX	Testes*+	X	Mammary gland*
	Pancreas*	X	Epididymides	X	Parathyroids*
		X	Prostate	X	Thyroids*
	RESPIRATORY	X	Seminal vesicle		
X	Trachea*	XX	Ovaries* +(with oviducts)		OTHER
X	Lung*	X	Uterus*	X	Bone*
	Nose	X	Cervix	X	Skeletal muscle*
	Pharynx	X	Vagina	X	Skin*
	Larynx			X	All gross lesions and masses*

*Required for chronic toxicity and chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.
+Organ weight required in chronic toxicity studies.

II. RESULTS

A. Observations

1. Toxicity

Clinical signs indicative of a test substance-related effect were not observed at an increased incidence in the treated groups compared to the control, were not present in a concentration-effect related manner, and/or were the types of abnormalities commonly observed in long-term studies with rats

Palpable masses were increased compared to those of diet control and placebo control group animals (22/70 and 21/70, respectively) for males in the 0.5 and 2.5 ppm groups (26/50 and 30/50, respectively). These incidences were significantly increased at $p < 0.05$ using the Fisher's Exact Test. The incidence of multiple masses was also significantly increased in the 0.5 and 2.5 ppm group males (12/50 both groups) compared to the placebo control group (7/70); this relationship was significant at $p< 0.05$ using Fisher's Exact Test as well. This increased incidence in palpable masses was not observed in either the 50 or the 250 ppm groups, consequently, the increases observed in the 0.5 and 2.5 ppm groups could not be attributed to the administration of methyl bromide.

2. Mortality

Survival data are summarized in Table 2. At the 52-week interim sacrifice, no test substance-related effect was evident; survival for any treated or control group remained at or above 90 %.

For the main study, male rats fed the 2.5 ppm diet had significantly decreased ($p < 0.05$, RXC Chi-Square Test) survival (15/50) when compared to the diet only control group. Males fed the 0.50 ppm diet also had a low survival rate (16/50) compared to the diet control (26/50), however, this was not a statistically significant relationship. The survival of the placebo control was similar to the 2.5 ppm treated group and the survival in the 50 and 250 ppm groups was not different from the controls. Survival of the treated females in the oncongenicity study was similar to both the diet and placebo control groups.

TABLE 2. Percent survival of male and female rats fed methyl bromide for up to 104 weeks.

Phase	Dietary concentration (ppm)					
	0 (Diet)	0 (Placebo)	0.50	2.5	50	250
Male						
Interim	100	100	NA	NA	100	100
Main Study	52	34	32	30	44	58
Female						
Interim	90	100	NA	NA	90	100
Main Study	40	38	44	48	56	58

Data taken from p. 38, MRID 44462501.

B. Body weight and body weight gain

Selected mean body weights are summarized in Table 3. Statistically significant decreases in mean body weights were observed for the males and females treated with 250 ppm methyl bromide. These decreases were evident beginning week 1 of the study and continuing through week 81 for males or week 63 for females when compared to either control group. Males in the 250 ppm group had a decrease of 5.5% in mean body weight compared to the diet control at week 2, by week 14 this decrease was 10% and remained consistently lower through week 70. This effect gradually disappeared as these animals gained weight and the mean body weights of males in the 250 ppm group were comparable to the diet and placebo control values by week 104. Females in the 250 ppm group had a decrease of 3.7% in mean body weight compared to the diet control at week 2, by week 14 this decrease was 8.3% and also remained consistently lower through week 57. After week 57 females in the 250 ppm group gained weight gradually and the decreases disappeared by the end of the study (week 104) at which time this group had mean body weight values that were similar to controls. Other treated groups (0.50, 2.5, and 50 ppm) had mean body weights that were comparable to controls throughout the study.

Selected intervals of mean body weight gain are presented in Table 4. Mean body weight gain was markedly decreased during the first 18 months of the study for animals treated with 250 ppm methyl bromide; decreases of 9-18% and 12-21% were observed for males, and 7-22% and 11-19% were observed for females when compared to the basal diet and placebo control groups, respectively. For the first 13 weeks of the study, males in the 250 ppm group had a decrease of about 15.2 and 17.9 % compared to the diet and placebo controls, respectively. At the end of the first year, these decreases for males treated with 250 ppm were 16.0 to 18.2 % compared to the diet and placebo controls, respectively. After week 52, these decreases gradually disappeared. Through week 13 of the study, females treated with 250 ppm had decreased body weight gains of 16.7 and 17.3 % for the diet and placebo control groups, respectively. These decreases gradually declined throughout the course of the study and were similar to control values at the end of the study (week 104).

Some sporadic increases and decreases in mean body weight gain were noted in the 0.5, 2.5, and 50 ppm groups compared to the controls throughout the study. As these changes did not appear to be concentration-dependent, they were not considered to be test substance-related.

TABLE 3. Selected mean body weights (g) in male and female rats fed methyl bromide for up to 104 weeks

Week	Dietary concentration (ppm)					
	0 Diet	0 Placebo	0.5	2.5	50	250
Male						
1	249.0	252.0	256.0	252.0	250.0	242.0*
8	441.0	451.0	456.0	449.0	441.0	404.0**
13	499.0	508.0	513.0	504.0	495.0	453.0**
26	580.0	589.0	600.0	589.0	575.0	521.0**
39	633.0	649.0	659.0	646.0	629.0	568.0**
52	670.0	683.0	697.0	684.0	661.0	595.0**
65	719.0	721.0	753.0	742.0	711.0	656.0**
78	743.0	760.0	773.0	762.0	737.0	691.0*
91	761.0	735.0	774.0	781.0	774.0	722.0
104	690.0	685.0	725.0	673.0	667.0	700.0
Female						
1	171.0	171.0	169.0	170.0	173.0	166.0
8	252.0	253.0	251.0	250.0	255.0	235.0**
13	275.0	276.0	273.0	270.0	275.0	253.0**
26	303.0	305.0	303.0	300.0	305.0	281.0**
39	332.0	336.0	335.0	334.0	334.0	309.0**
52	355.0	360.0	359.0	353.0	359.0	330.0**
65	396.0	403.0	399.0	396.0	405.0	375.0
78	445.0	462.0	449.0	443.0	465.0	418.0
91	479.0	501.0	469.0	464.0	519.0	446.0
104	467.0	488.0	455.0	445.0	489.0	454.0

Data taken from Table 5, pp. 107-150, MRID 44462501.
* Significantly different from the placebo group at $p < 0.05$ using Dunnett's test
**Significantly different from the diet and placebo groups at $p < 0.01$ using Dunnett's test

TABLE 4. Mean body weight gain (g) for selected weeks in male and female rats fed methyl bromide for up to 104 weeks

Week	Dietary concentration (ppm)					
	0 Diet	0 Placebo	0.5	2.5	50	250
Male						
0-13	303.0	313.0	313.0	307.0	299.0	257.0**
14-26	71.0	70.0	77.0	75.0	71.0	62.0*
27-39	45.0[+]	53.0*	50	52.0	46.0	39.0[‡]
53-65	45.0	42.0	48.0	51.0	48.0	51.0
92-104	-79.0	-88.0	-55.0	-110.0	-116.0	-37.0
Female						
0-13	126.0	127.0	127.0	122.0	125.0	105.0**
27-39	26.0	27.0	27.0	29.0	24.0	24.0
53-65	28.0	29.0	38.0	38.0	39.0	36.0
92-104	-29.0	-30.0	-43.0	-37.0	-30.0	-6.0

Data taken from Table 6B. pp. 237-240, MRID 44462501
* Significantly different from the diet control at $p < 0.05$ using Dunnett's Test.
**Significantly different from the diet and placebo controls at $p < 0.01$, using Dunnett's Test.
[+] Significantly different from the placebo control at $p < 0.05$, using Dunnett's Test.
[‡] Significantly different from the placebo control at $p < 0.01$, using Dunnett's Test.

C. Food consumption and compound intake

1. Food consumption

Food consumption and food utilization data are summarized in Table 5. Male and female rats fed the 250 ppm diet consumed significantly ($p < 0.05$ or $p < 0.01$) less food than the diet or placebo control groups through week 72 for males or week 55 for females. Males receiving 250 ppm had decreased food consumption that ranged from 3.7 - 11.5 % through week 71-72, and females at this concentration had decreases of 4.8 - 10.5% through week 54-55 compared to their respective control groups. The 0.5, 2.5, and 50 ppm groups were not observed to have test substance-related effects on food consumption. There were some sporadic significant differences present throughout the study, however, these were not present in a dose-related manner and/or a trend was not present.

TABLE 5. Mean food consumption (g/kg/day) for selected weeks in male and female rats fed methyl bromide for up to 104 weeks

Week	Dietary concentration (ppm)					
	0 Diet	0 Placebo	0.5	2.5	50	250
Male						
0-1	25	26	26	26	25	23**
10-11	27	27	28++	28++	27	26**
20-21	26	26	26	26	25	23**
50-51	26	26	27	27	26	24**
70-71	27	28	29	27	28	25 +
80-81	27	27	27	27	27	27
90-91	27	24	27	26	28	26
103-104	22	25	25	19	23	23
Female						
0-1	18	18	18	18	19	17**
12-13	20	20	20	20	20	19**
26-27	20	20	20	20	19	18**
45-46	20	21	20	20	20	19**
50-51	20	21	20	20	20	20**
70-71	23	23	22	22	22	21
90-91	21	21	20	21	23	22
103-104	18	20	20	19	19	19

Data taken from Table 6B, pp. 241-284, MRID 44462501

**Significantly different from the diet and placebo controls at $p < 0.01$, using Dunnett's Test.
+ Significantly different from the placebo control at $p < 0.05$, using Dunnett's Test.
++ Significantly different from the diet control at $p < 0.05$, using Dunnett's Test.

2. Compound consumption

The average consumption of test material at each dietary concentration is presented in Table 1.

D. Ophthalmoscopic examination

No treatment-related effects were observed on the eyes of male or female rats fed Methyl Bromide for up to 104 weeks.

E. Blood work

1. Hematology

There were no test substance-related effects on hematological parameters. However, there were a few sporadic changes in some parameters compared to the diet and/or the basal controls. In the 50 ppm group males there was a decreased mean platelet count and the 2.5 ppm female group had a decreased mean MCH at the week 25 evaluation. There were a few changes noted at the 104 week evaluation as well, including decreased mean erythrocyte count and hematocrit values among males in the 2.5 and 50 ppm groups and a decreased mean hemoglobin value for the 2.5 ppm group males. Also at the 104 week evaluation, the 50 ppm group males had an increased mean neutrophil differential (%) count and the 0.5 ppm group females had a decreased mean platelet count. Although these changes were statistically significant compared to one or both control, none of these differences were observed to be concentration-dependent or biologically significant.

2. Clinical chemistry

Sodium was slightly increased compared to one or both the control groups in the 2.5, 50, and 250 ppm group males at the week 52 evaluation. At the week 104 evaluation, sodium was increased in the 50 and 250 ppm group males and in the 50 ppm group females. There were increased chloride levels in the 250 ppm males and females at the 52 week evaluation. These electrolyte changes were less than 1% and therefore were not biologically significant. Also at the 52 week evaluation there were increased alanine aminotransferase and gamma glutamyl transferase levels in the 50 ppm group males. Calcium levels were increased in the 50 and 250 ppm group males, potassium levels were decreased in the 50 ppm group males, and albumin was increased in the 50 ppm group females at the week 77 evaluation. Finally, at the 104 week evaluation, the A/G ratio was decreased in the 50 ppm group males, and globulin was increased in the 0.5 ppm group males. These alterations were statistically significant compared to one or both controls but were considered to be within the realm of acceptable biological variability and therefore not considered to be test substance-related.

F. Urinalysis

Males in the 50 ppm group had increased urine output compared to the controls ($p<0.05$) only at the week 103 evaluation. There were no other differences observed for any of the treated groups compared to the controls.

H. Sacrifice and pathology

1. Organ weight

There were some sporadic, minor changes in organ weights compared to the controls. At the 52-week interim necropsy, there were decreased mean absolute kidney and liver weights and increased mean testes and brain weights relative to the final body weight for males in the 250 ppm group, these differences were significantly different from both control groups at $p<0.05$ or $p<0.01$. At the final necropsy, the 250 ppm females had significantly reduced mean absolute kidney weights compared to the diet control. No other significant differences between controls and exposed groups were observed at either sacrifice.

2. Gross pathology

The incidences of gross findings in animals assigned to the interim group (52-week sacrifice) were similar in treated and control groups and/or were common findings for animals in this type of laboratory setting. This was also true for the animals terminated at the end of the study and the animals that were found dead or sacrificed *in extremis*.

3. Microscopic pathology

a) Non-neoplastic – Microscopic findings in all animals were similar and there were no test substance-related abnormalities observed for any group. At the 52-week sacrifice, abnormalities such as adrenal cystic degeneration, nephrophathy, spongiosis hepatitis, and nonsuppurative inflammation were considered to be age-related changes or secondary to pre-existing aging lesions. These lesions were also observed at similar incidence in the control groups. This was also true for the findings at the 104-week sacrifice. Lesions such as nephropathy, suppurative infalmmation, cardiomyopathy, stromal fibrosis of the uterus and/or cervix were observed at similar frequencies in all treated and control groups.

b) Neoplastic – No treatment-related neoplastic findings were observed in male or female rats receiving the test material. The most frequently observed neoplasm was adenoma of the pars distalis portion of the pituitary gland. Mammary tumors were frequently observed in females rats and contributed to the death of some animals.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that administration of Methyl Bromide for up to 2 years caused adverse effects at 250 ppm. These included changes in body weight and food consumption. The no-observed-adverse-effect level (NOAEL) was 50 ppm. The study authors also concluded that administration of Methyl Bromide was not associated with an oncogenic effect.

B. Reviewer's Discussion

Survival was not effected by administration of methyl bromide in this study. There were no significant differences between control and treated groups in survival for either the interim sacrifice or the main study.

There were no increased incidences of clinical signs that could be attributed to the administration of the test substance. In the 0.5 and 2.5 ppm male groups, there was an increased incidence of palpable masses compared to the diet and placebo control groups. This effect was not attributed to the test substance since it did not occur in a dose-related manner and other related parameters such as survival were not effected.

Body weights were significantly affected in males and females administered the 250 ppm diets. During the first year of the study, the body weights and body weight gain of males and females treated with 250 ppm were significantly decreased compared to the controls. These differences gradually disappeared during the second year of the study and by the terminal sacrifice, there were no differences between treated and control groups. Decreased food consumption was also observed for these groups during this time period. Therefore these differences in body weight and body weight gain can be attributed, at least in part, to the decreased food consumption in these groups.

Treatment with methyl bromide was not associated with significant hematological changes. Only a few spurious changes were observed, and these were neither consistent nor concentration-related.

Clinical chemistry parameters showed only a few sporadic statistically significant changes at different time points during treatment. Therefore, the reviewer does not consider these changes such as increased sodium and chloride or increased liver enzymes to be treatment related. Other statistically significant changes were observed in clinical chemistry parameters, but are not considered treatment related, because the changes were transient, the magnitude of the changes were small, or no dose-response relationship was observed.

Kidney and liver weights for males in the 250 ppm group were decreased compared to controls at the 52-week sacrifice. This was considered to be related to reduced final body weight at this interval because the relative weights of these organs were similar to controls and no accompanying histopathological findings were detected. Females in the 250 ppm group at the terminal sacrifice (104-weeks) were observed to have a statistically significant decrease in mean absolute kidney weight compared to the diet control only. Relative organ weight however, was not reduced and no indications of renal damage were observed in conjunction with this effect. This effect was attributed to the decreased mean body weights in this group during the first year of the study.

Incidences of gross and microscopic findings attributable to methyl bromide administration were not observed in male or female rats. Abnormal observations were not considered treatment related because they were transient, common age-related changes in rats, and/or occurred with same incidence in controls.

Therefore, treatment with methyl bromide caused decreases in body weights, body weight gain, and food consumption at 250 ppm. The lowest-observed-adverse-effect level (LOAEL) is 250 ppm (based on decreased body weight and body weight gain in male and female rats. The corresponding no-observed-adverse-effect level (NOAEL) is 50 ppm.

Feeding of methyl bromide for up to 104 weeks did not result in a statistically significant increase in the incidence of neoplastic lesions at any anatomical site. Dosing was considered adequate based on decreases in body weight and body weight gain in males and females.

C. Study Deficiencies

The concentration of Formulation I of methyl bromide after storage at -15 °C for 24 months was decreased approximately 20% compared to the time zero concentration. In the worst case scenario, the rats in the two lower dose groups (0.5 and 2.5 ppm groups) could have been dosed at only 80% of the target concentration for the last year of the study. This decrease in concentration was not considered to have significantly affected the outcome of the study.